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TRANSCRANIAL DIRECT CURRENT STIMULATION DOES NOT MODULATE MOTOR CORTEX EXCITABILITY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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ABSTRACT: *Introduction:* Amyotrophic lateral sclerosis (ALS) is a progressive disease caused by the degeneration of upper and lower motor neurons. The etiology of ALS is unclear, but there is evidence that loss of cortical inhibition could be related to motor neuron degeneration. We sought to determine whether cathodal transcranial direct current stimulation (tDCS) can reduce cortical excitability in patients with ALS. *Methods:* Three sessions of cathodal tDCS, lasting 7, 11, or 15 minutes, were performed in 10 patients and 10 healthy controls. Corticospinal excitability was measured before and after the tDCS. *Results:* Cathodal tDCS induced a consistent decrease in corticospinal excitability in healthy controls, but not in ALS patients. *Conclusions:* The failure of tDCS to produce an excitability shift in the patients supports the potential diagnostic value of tDCS as a marker of upper motor neuron involvement. However, variation in corticospinal excitability measurements both inter- and intraindividually will limit its usefulness.

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease caused by the degeneration of both the upper and lower motor neurons that control voluntary muscle movement. Although the exact etiology of ALS is unclear, loss of inhibition in motor cortex circuits has been described in patients with ALS, particularly early in the disease.[1] It is speculated that loss of inhibition not only causes central motor neuron loss but also drives anterior horn cells into metabolic deficit, a process called anterograde degeneration.[2]

A decade ago, a non-invasive tool to modulate cortical excitability, transcranial direct current stimulation (tDCS), was reintroduced.[3] With tDCS, a weak constant electrical current ($\leq$1 mA), which passes through the skull and underlying structures to the cortical structures, up- or downregulates cortical excitability depending on the stimulation polarity used. Cathodal tDCS over the motor cortex, where the cathode is placed over the primary motor cortex and the anode above the contralateral eyebrow, leads to decreased excitability of the motor cortex in healthy controls, evidenced by decreased muscle responses elicited by transcranial magnetic stimulation (TMS).[4–17] If tDCS is applied for several minutes, the changes can outlast the stimulation by up to 1 hour.[11,18] Given cortical disinhibition in patients with ALS, cathodal tDCS is considered a proposed treatment option. In healthy subjects, stimulation for at least 3 minutes at 1 mA already elicits an after-effect.[10] Stimulation for up to 15 minutes at 1 mA is without noticeable side effects.[19] Thus, stimulation for 3–15 minutes appears to be safe and effective.

Only one study has investigated the effects of tDCS stimulation in patients with ALS.[6] Anodal and cathodal tDCS, performed for 7 minutes, led to a consistent modification of cortical excitability in healthy subjects, but not in patients with ALS. However, in this study the duration of tDCS stimulation was not varied, even though studies of healthy individuals have shown that the duration of stimulation influences the extent and duration of cortical modulation.[11,18] The investigators suggested that tDCS might be useful as a diagnostic tool for ALS. They did not discuss the potential of tDCS as a therapeutic strategy. Obviously, to have a therapeutic effect on the continuous process of anterograde degeneration, cortical modulation needs to be present, but it also must be long-lasting.

The first aim of our study was to address the potential of tDCS as a therapeutic strategy. The second aim was to further investigate the diagnostic potential of short-duration tDCS, as reported by Quartarone et al.[6] For this purpose, we studied the effect of lengthening the tDCS stimulation up to 15 minutes in an attempt to induce lasting changes in cortical excitability.

METHODS

Subjects. Ten patients with sporadic ALS and 10 healthy controls participated in this study. All

Abbreviations: ADM, abductor digiti minimi; ALS, amyotrophic lateral sclerosis; ALS-FSR-R, ALS Functional Rating Scale, revised; ANOVA, analysis of variance; CMAP, compound muscle action potential; EMG, electromyography; ICF, intracortical facilitation; MEP, motor-evoked potential; $SI_{0.5mV}$, stimulator intensity to induce 0.5-mV MEPs; SI_{1mV}, stimulator intensity to induce 1-mV MEPs; SICI, short-latency intracortical inhibition; tDCS, transcranial direct current stimulation; TMS, transcranial magnetic stimulation

Key words: amyotrophic lateral sclerosis, cortical excitability, paired-pulse TMS, transcranial direct current stimulation, transcranial magnetic stimulation

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patients were categorized as having clinically probable ALS according to the revised El Escorial criteria.[20] In all patients and controls we were able to consistently elicit MEPs in the contralateral target muscle with a mean peak-to-peak amplitude of at least 1 mV. At the time of the study all patients were on riluzole. The patients were recruited from the National ALS Center. The controls were recruited through posters and flyers displayed at the Radboud University Nijmegen Medical Center.

All patients and controls gave written informed consent prior to inclusion in the study. The study was approved by the ethics committee of the Radboud University Nijmegen Medical Center and was performed in accordance with the ethics standards established by the Declaration of Helsinki.

Transcranial Direct Current Stimulation. The study protocol consisted of three experimental sessions separated by 1 week. In each session, participants received cathodal tDCS (1 mA) for either 7, 11, or 15 minutes (in random order). We did not use anodal tDCS, because only cathodal tDCS is expected to be potentially effective in patients with ALS. tDCS was delivered using a constant-current stimulator (Eldith, NeuroConn GmbH, Ilmenau, Germany) via two conductive rubber electrodes (35 cm^2) inside saline-soaked sponges placed on the scalp. The cathode was placed over the left primary motor hand area and the anode above the right eyebrow. Before the electrodes were placed, the skin was rigorously cleaned and lightly abraded to reduce impedance. The target skin impedance, as measured by the stimulator, was <15 kΩ. To avoid abrupt sensations, the stimulation period was initiated by a fade-in period (10 seconds) and completed by a fade-out period (10 seconds).

Transcranial Magnetic Stimulation. We used various well-established TMS paradigms[21] to compare corticospinal excitability of the stimulated left primary motor hand area before (baseline, 0) and 5 and 20 minutes after tDCS. All TMS measurements were performed using two monophasic magnetic stimulators (Magstim 200^2; Magstim Co., Whitland, Wales, UK), which were connected through a user interface module (BiStim^2) to a standard circular coil (diameter 90 mm, Magstim) centered above the vertex with the A-side visible. Each stimulus induces an anticlockwise current, resulting in posterior–anterior current flow in the left hemisphere. Because of non-focal stimulation with tDCS, we measured excitability with a round non-focal coil. Several measures of corticospinal excitability were assessed with single- and paired-pulse TMS.

(i) *Stimulus intensity needed to evoke a motor-evoked potential of 0.5-mV amplitude ($SI_{0.5mV}$).* $SI_{0.5mV}$ was defined as the lowest stimulator output intensity at which a single TMS pulse induced motor-evoked potentials (MEPs) of at least 0.5-mV peak-to-peak amplitude in the right abductor digiti minimi (ADM) muscle in at least 5 of 10 trials. We used this measure instead of the resting motor threshold (criterion of 50 μV), because patients often had fasciculations in their hand muscles. The presence of fasciculations rendered it impossible to distinguish between small MEPs and spontaneous fasciculations when TMS was given around threshold intensity.

(ii) *Single-pulse MEPs.* Before tDCS, the lowest stimulator output intensity needed to induce MEPs with a mean amplitude of approximately 1 mV (SI_{1mV}) was determined from, on average, 20 consecutive trials. This intensity was used to deliver 30 consecutive pulses at, on average, 0.25 Hz (random 4-, 5-, and 6-second intervals).

(iii) *Paired-pulse TMS* was performed for each subject to investigate short-interval intracortical inhibition and facilitation (SICI and ICF, respectively).[22] The conditioning subthreshold stimulus was set to 80% of the $SI_{0.5mV}$ and was delivered through the same magnetic coil at interstimulus intervals of 2–3 ms to assess SICI, and 10 and 12 ms to assess ICF before a suprathreshold test stimulus. The test stimulus intensity was set to SI_{1mV} and was kept constant throughout the experiment. This procedure allows the measurement of intracortical inhibition and facilitation, which are considered to reflect the excitability of short inhibitory and facilitatory interneuronal circuits in the motor cortex.[23] A randomized protocol was run to measure SICI and ICF. It consisted of 50 stimuli given at, on average, 0.25 Hz in blocks of 10 stimuli. Forty conditioned MEPs were recorded (10 for each ISI) and 10 unconditioned MEPs.

Procedure. During each session, the participants were seated in a slightly reclining chair with the elbow semiflexed and the forearm supinated, fully relaxed, and supported by a pillow on the thigh. Prior to the TMS baseline measurements, compound muscle action potentials (CMAPs) were measured in the ADM and the abductor pollicis brevis (APB) muscles of the right hand through supramaximal peripheral stimulation of the ulnar and median nerve (6 cm proximal to the active electrodes), respectively. Stimulation was done using a constant-current stimulator (Model DS7A; Digitimer, Ltd., Welwyn Garden City, UK).

We used visual electromyographic (EMG) feedback to be sure of complete relaxation of the ADM muscle. No feedback was given for the other hand muscles. We chose the ADM muscle because other commonly used muscles, such as the first dorsal interosseus muscle and APB, are the most atrophic

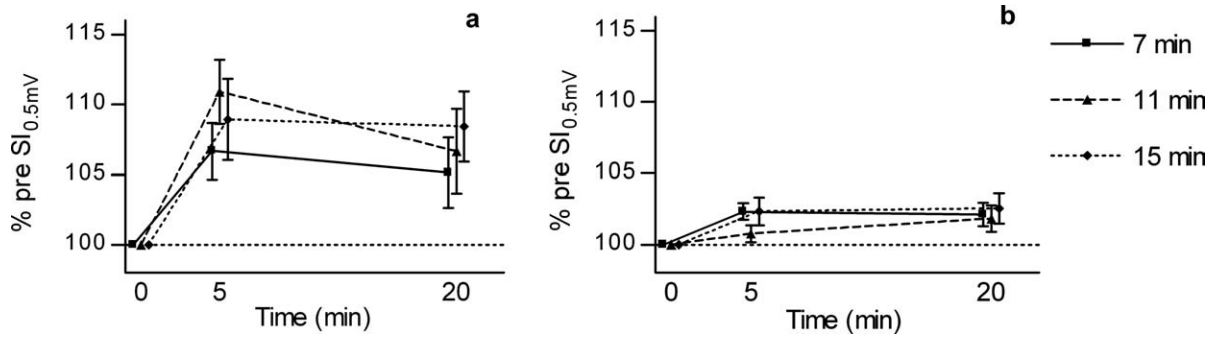

FIGURE 1. $SI_{0.5mV}$ stimulus intensities given as percentage of the pre-tDCS (0 minute) control values for healthy controls **(a)** and ALS patients **(b)** at two time-points (5 and 20 minutes) after 7 (squares with solid line), 11 (triangles with dashed line), and 15 (diamonds with dotted line) minutes of tDCS. The error bars signify the standard error of the mean.

in patients with ALS (split hand[24,25]), which makes it more difficult to evoke consistent MEPs in those muscles. Obtaining the excitability measures took, on average, 10 minutes, and one complete session took 65 minutes.

Data Acquisition. Surface EMG activity of the ADM muscle was recorded using self-adhesive Ag–AgCl surface electrodes (Soft-E H69P; Kendall-LTP, Chicopee, Massachusetts) using a belly–tendon montage. EMG signals were amplified (0.6 μV/bit) and bandpass filtered between 10 and 500 Hz. The EMG signals were acquired at a rate of 10 kHz (CED 1401 Laboratory Interface; Cambridge Electronic Design, Cambridge, UK) and recorded using Spike2 software (Cambridge). Digitized recordings, running from 500 ms before to 1500 ms after each TMS trigger, were stored for further analysis.

Analysis. For each block of measurements [baseline (0), 5 and 20 minutes after tDCS], the peak-to-peak amplitude of each MEP (in millivolts) was measured off-line, and the mean MEP amplitude was calculated for each stimulation condition (single-pulse MEP, SICI, and ICF) with custom-written MatLab (The MathWorks, Inc., Natick, Massachusetts) software scripts. To compare the responses of the individuals, the baseline values of $SI_{0.5mV}$ and single-pulse MEPs were set to 100%, and for the follow-up measurements the relative change was calculated.

For SICI and ICF, the ratio between the conditioned MEP and the unconditioned MEP was calculated from individual data. The SICI was calculated as the mean of ISI 2 ms and 3 ms, and ICF as the mean of ISI 10 ms and 12 ms. Ratios <1 indicate inhibition, whereas ratios >1 indicate facilitation.

Stimulus intensities and MEP amplitudes for the different excitability measures were entered separately in three-way repeated-measures analyses of variance (ANOVAs) with tDCS duration (7, 11, and 15 minutes) and time [baseline (0), 5, and 20 minutes after tDCS] as within-subject factor and group (patients or controls) as between-subjects factor. The Greenhouse–Geisser method was used in case of non-sphericity. If the *F*-value was significant, paired-sample, two-tailed *t*-tests were used for post hoc comparisons. For all tests, $P \leq 0.05$ was considered significant. Data are given as mean $\pm$ standard error of the mean, unless otherwise indicated.

RESULTS

Subjects. The 10 healthy controls were well matched with the 10 patients for age (ALS mean: 54.0 $\pm$ 3.1 years; controls: mean 57.2 $\pm$ 1.6 years; $P = 0.373$) and gender (ALS: 6 males; controls: 7 males; chi-square test: $P = 0.639$). The mean disease duration in patients was 24.2 $\pm$ 4.2 months. The mean score on the revised ALS Functional Rating Scale (ALS-FRS-R)[26] was 36.6 $\pm$ 1.5. None of the 20 subjects reported adverse effects during or after the experiments. The tDCS stimulation was neither painful nor unpleasant for either the healthy controls or the patients.

Maximal CMAP amplitude of the ADM was similar in the patients and controls (11.1 $\pm$ 0.8 mV and 12.8 $\pm$ 0.5 mV, respectively; $P = 0.330$), whereas CMAP amplitude of the APB was significantly lower in the patients than in controls (4.7 $\pm$ 0.5 mV and 8.4 $\pm$ 0.7 mV, respectively; $P = 0.035$). Neither the $SI_{0.5mV}$ ($P = 0.96$) nor the SI_{1mV} ($P = 0.86$) were significantly different between patients and controls.

Stimulus Intensity for Evoking MEPs of 0.5-mV Amplitude. Using $SI_{0.5mV}$ as the dependent variable, repeated-measures ANOVA revealed an effect of time ($F = 15.38$, $P > 0.001$), but not of tDCS duration. There was also a time $\times$ group interaction ($F = 6.01$, $P = 0.006$), indicating a difference in the responsiveness to tDCS between groups. In addition, a significant effect in the between-subject variable group was found ($F = 7.265$, $P = 0.015$). Post hoc paired *t*-tests demonstrated that $SI_{0.5mV}$ was

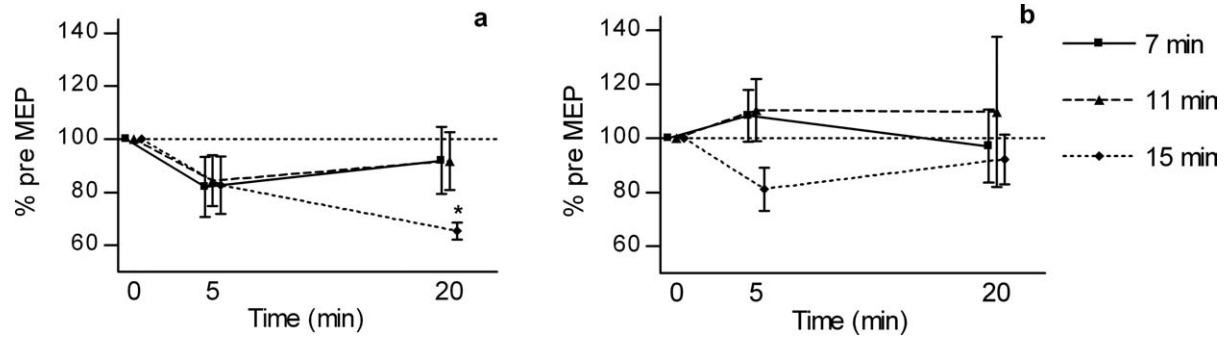

FIGURE 2. The single-pulse MEP amplitudes given as percentage of the pre-tDCS (0 minute) control values for healthy controls **(a)** and ALS patients **(b)** at two time-points (5 and 20 minutes) after 7 (squares with solid line), 11 (triangles with dashed line), and 15 (diamonds with dotted line) minutes of tDCS. The error bars signify the standard error of the mean.

increased at 5 minutes after both 7 and 11 minutes of tDCS ($P = 0.043$ and 0.008, respectively) and at 20 minutes after 15 minutes of tDCS ($P = 0.040$) in healthy controls (Fig. 1a). In patients, the tDCS effects on $SI_{0.5mV}$ were inconsistent. There was only an increase in $SI_{0.5mV}$ after 7 minutes of tDCS ($P = 0.02$) but not after 11 or 15 minutes of tDCS at 5 minutes after tDCS (Fig. 1b).

Single-Pulse MEPs. With regard to SI_{1mV} MEP amplitude, repeated-measures ANOVA revealed no significant effect of tDCS duration or time, and no interactions with group (Fig. 2). Obviously, post hoc analysis did show a significant decrease in MEP size after 15 minutes in healthy controls (Fig. 2a).

Intracortical Paired-Pulse Inhibition and Facilitation. Figures 3 (SICI) and 4 (ICF) show the paired-pulse data for healthy controls (Figs. 3a and 4a) and ALS patients (Figs. 3b and 4b). The baseline values of SICI and ICF were similar between the groups ($P = 0.517$ and 0.107, respectively). Repeated-measures ANOVAs of the SICI revealed a time × group interaction ($F = 4.80$, $P = 0.032$), indicating that tDCS had different effects on changes in SICI over time when comparing patients and controls. Although not significant for any of the tDCS durations, a reduction in SICI in healthy controls could be observed, whereas the patients with ALS showed no change or only a slight change in SICI. ANOVA of the ICF revealed a significant effect for the between-subject factor group ($F = 5.805$, $P = 0.027$). On post hoc testing, the ICF was higher overall in healthy controls compared with the ALS patients ($P < 0.01$). No effect of tDCS duration, time, or interactions with group was found for the ICF.

DISCUSSION

Even after 15 minutes of stimulation, cathodal tDCS does not induce a decrease of cortical excitability in patients with ALS. This is in clear contrast to the results in healthy controls that do show a decrease of cortical excitability with lengthening of the stimulation duration. These results are not encouraging for a potential therapeutic effect of tDCS. However, they confirm and extend the conclusion of the only other study that addressed the effect of tDCS in ALS. In their investigation, Quartarone et al. extensively discussed the potential mechanisms that could underlie the lack of responsiveness in the patient group, for example, anatomical alterations of the motor cortex and altered glutamate transmission.[6] They considered

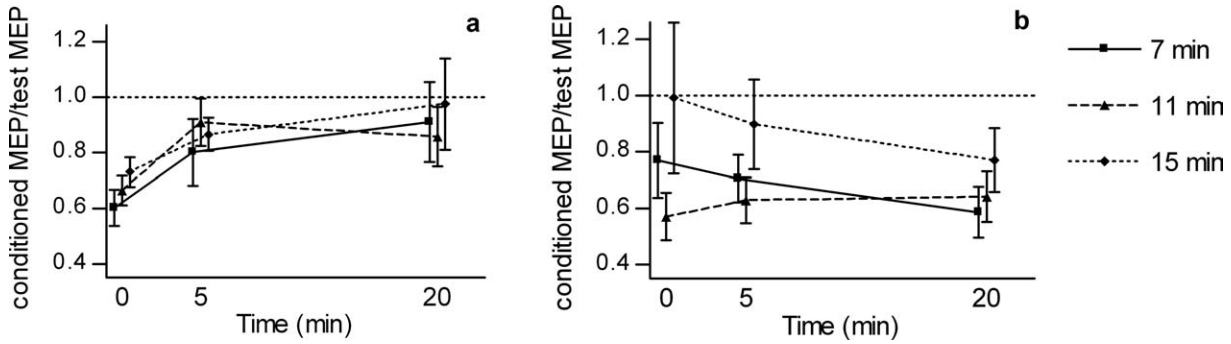

FIGURE 3. The short-interval intracortical inhibition (SICI) data given as ratio between the conditioned MEP and the unconditioned MEP amplitudes for healthy controls **(a)** and ALS patients **(b)** before (0 minute) and at the two time-points (5 and 20 minutes) after 7 (squares with solid line), 11 (triangles with dashed line), and 15 (diamonds with dotted line) minutes of tDCS. The error bars signify the standard error of the mean.

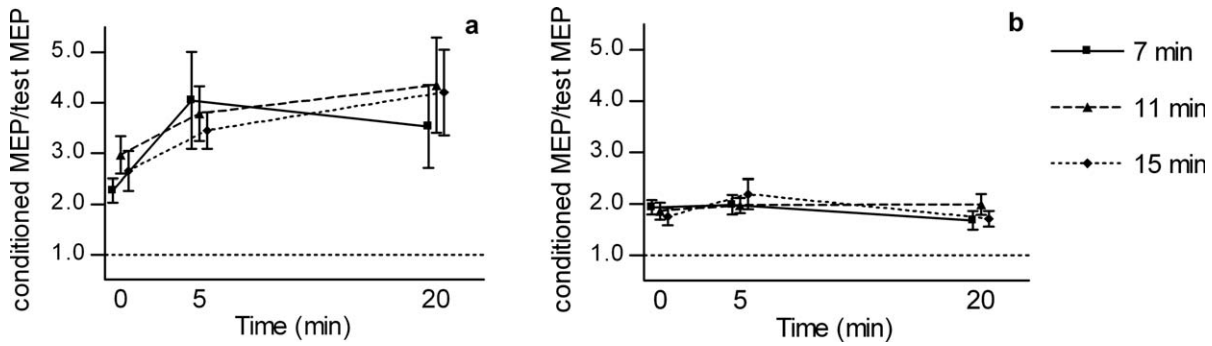

FIGURE 4. The intracortical facilitation (ICF) data given as ratio between the conditioned MEP and the unconditioned MEP for healthy controls **(a)** and ALS patients **(b)** before (0 minute) and at the two time-points (5 and 20 minutes) after 7 (squares with solid lines), 11 (triangles with with dashed lines), and 15 (diamonds with dotted lines) minutes of tDCS. The error bars signify the standard error of the mean.

that the threshold (duration of tDCS application) for induction of the tDCS effects could be higher in ALS patients compared with controls and that this possible explanation could have been excluded by applying longer duration tDCS protocols. Our study, in which we doubled the stimulation duration, now indeed excludes this possibility. A ceiling effect of MEP amplitude related to the loss of cortical neurons could be another explanation, but this appears unlikely, because in the study by Quartarone et al. the patients were in an earlier stage of disease. Also, the use of riluzole could have influenced the abnormal tDCS response,[27] but this is unlikely. In the Quartarone et al. study only 1 patient was on riluzole. The lack of tDCS after-effects in patients with ALS could also be related to pathological changes in upper motor neuron membrane function.

For now, we can only speculate on the implications of these results for the underlying pathological upper motor neuron degeneration. Although cathodal tDCS showed decreased relative glutamate levels and gamma-aminobutyric acid in the motor cortex in healthy controls,[28] in this study we only assessed the excitability with TMS. In other studies, repetitive TMS (or theta burst stimulation) was used to change the cortical excitability in ALS.[29,30] In those earlier studies cortical excitability measures were not performed and, ultimately, 1 year of treatment did not result in a reduced rate of deterioration in ALS patients.

Our study supports the suggestion by Quartarone et al.,[6] who indicated that an abnormal tDCS effect might be a neurophysiological feature of ALS. It raises the question of whether tDCS could be a diagnostic tool for ALS or for the detection of early upper motor neuron involvement in ALS. However, the large variability in the TMS responses with respect to the single MEP amplitudes, SICI and ICF, as described earlier,[31–33] which are not explained by age, gender, or disease duration (data not shown), will limit the diagnostic potential of the protocols applied.

We conclude that a single session of cathodal tDCS does not produce an excitability shift in patients with ALS. This is in contrast to the effect of tDCS in healthy controls, where tDCS can induce a decrease of cortical excitability. The variability in TMS effect that is found in patients with ALS hampers its utility as a diagnostic tool and, if diagnostic studies are considered, they should be performed strictly according to the STARD criteria.[34] Our results are not encouraging for the therapeutic effect of tDCS. However, further studies are warranted, because, to date, only "one-session tDCS" has been investigated, and repeated cathodal tDCS sessions may provide new insights.

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